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Phase-Switching Depolymerizable Poly(carbamate) Oligomers for Signal Amplification in Quantitative Time-Based Assays

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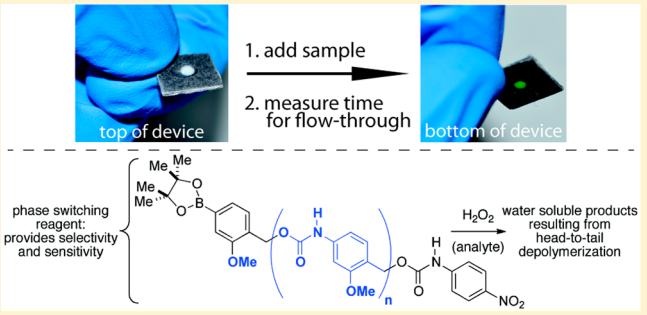
Supporting Information

ABSTRACT: This article describes the use of poly(carbamate) oligomers that depolymerize from head-to-tail as phase-switching reagents for increasing the sensitivity of quantitative point-of-care assays that are based on measurements of time. The poly(carbamate) oligomers selectively react with hydrogen peroxide (a model analyte) and provide sensitivity by depolymerizing in the presence of the analyte to convert from water-insoluble oligomers to water-soluble products. This switching reaction enables a sample to wick through a three-dimensional paper-based microfluidic device, where the flow-through time reflects the quantity of the analyte in the sample. Oligomers as short as octamers enable quantitative detection to low nanomolar concentrations of the analyte.



INTRODUCTION

A long-standing challenge in the area of point-of-care (POC) diagnostics has been the development of operationally simple and inexpensive platforms for conducting reproducible and rapid quantitative assays.[1−3] The ideal quantitative POC assay, particularly for use in resource-limited environments such as the developing world, should be inexpensive, be straightforward to operate, and provide rapid and reproducible quantitative results without the need for specialized electronic devices to measure the output of the assay.[4,5] As a step toward this goal, we recently described an approach for quantitative POC assays that requires only measurements of time as the readout (Figure 1).[6] The selectivity in the assay is based on a hydrophobic-to-hydrophilic switch of a hydrogen-peroxide-responsive small molecule (compound **1**, Figure 1d). This novel assay strategy, however, was limited in sensitivity (e.g., hydrogen peroxide was measured only to ∼3 mM levels) and thus was restricted in the types of applications to which it could be applied.

Herein we describe an approach for substantially improving the sensitivity of the model assay by using analyte-triggered oligomers, rather than small molecules, to achieve signal amplification in the assay. Specifically, we rationally designed water-insoluble poly(carbamate) oligomers that depolymerize from head-to-tail[7−10] to reveal water-soluble products in response to hydrogen peroxide, thus inducing a large hydrophobic-to-hydrophilic switch. This approach improves the sensitivity 4 orders of magnitude compared with our previous system (limits-of-detection of hydrogen peroxide are now 146 nM). Moreover, by optimizing the number of layers of paper containing the oligomer in the assay platform, we further improved the limit of detection by approximately another order of magnitude, providing a limit of detection of 6 nM and a ∼500 000-fold improvement over our previous system. These improvements in sensitivity provide an important step toward realizing a generalizable quantitative POC assay platform for use in resource-limited environments since many analytes of interest are present in samples at (or below) micro- and nanomolar levels.

RESULTS AND DISCUSSION

Design of the Assay Platform. Figure 1a provides an illustration of the assay platform. The assay is conducted in a three-dimensional (3D) paper-based microfluidic device that consists of stacked, alternating layers of (i) paper that has been patterned with wax into hydrophobic and hydrophilic regions, and (ii) double-sided adhesive tape, which has holes patterned into it using a CO_2 laser cutter.[11−13] The holes in the tape are filled with disks of hydrophilic paper such that hydrophilic regions in paper connect with the hydrophilic disks in the tape. The result is a disposable and inexpensive multilayered 3D device that wicks aqueous fluids into defined regions within the device. Each layer of the device (except layers 1, 4, and 7) is preloaded with a reagent and dried before assembly, such that addition of sample to the top of this device allows the assay to occur automatically without user intervention.

As the sample passes from layer 1 into layer 2, it redissolves HEPES buffer salts to control the pH of the sample. In layers 3 and 5, the sample encounters compound **1** (Figure 1d), which is hydrophobic and water-insoluble, and thus alters the wetting properties of the paper. In the absence of the analyte, the

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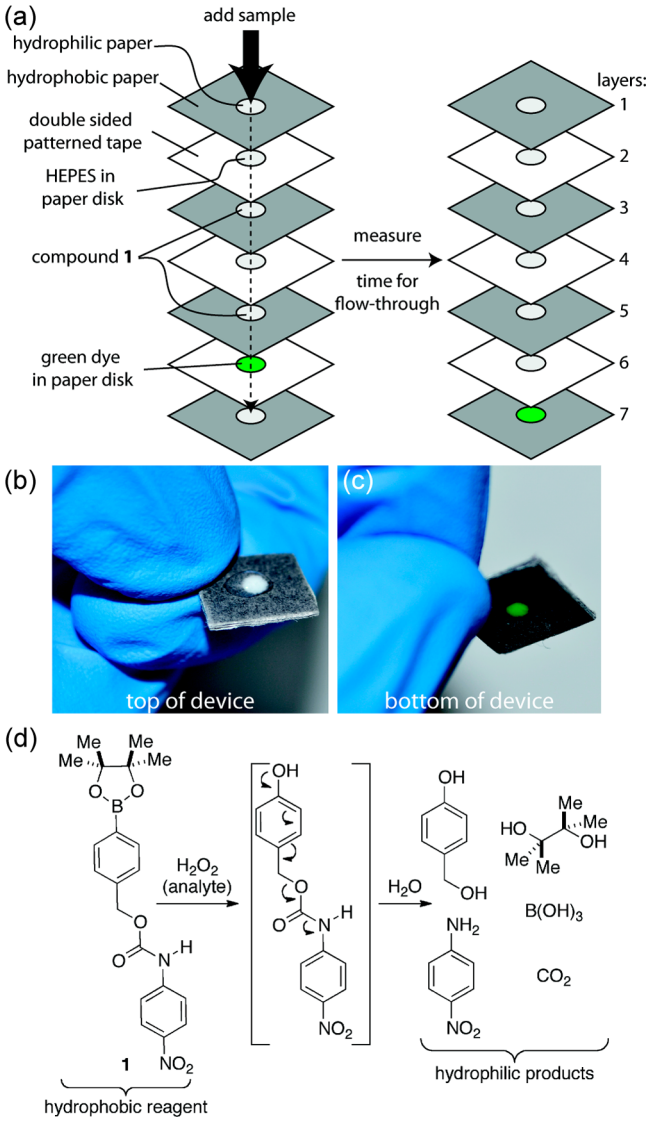

Figure 1. Strategy for obtaining quantitative assay results by measuring the time required for a sample to pass from top to bottom of a paper-based microfluidic device.[6] (a) Graphical representation of the device, including the dry reagents that are included in each layer. (b) Photographs of the top of the device (where the sample is added) and (c) the bottom (after the sample has redissolved the dried food coloring and filled the hydrophilic circle on layer 7 of the device). The dimensions of the devices are 1 cm ×1 cm ×0.9 mm. (d) Compound **1**, which is used in layers 3 and 5. Compound **1** converts from a hydrophobic molecule to hydrophilic molecules selectively in response to hydrogen peroxide (the model analyte) through a proposed quinone methide elimination mechanism.

sample stops wicking (or slowly wicks, depending on the quantity of **1** in the paper) through hydrophobic layers 3 and 5. In the presence of hydrogen peroxide, **1** selectively degrades into hydrophilic products (Figure 1d), thus switching phases from insoluble **1** into soluble products, and, consequently, changing the wetting properties of the paper back to hydrophilic. After this switching reaction occurs, the sample wicks to layer 6 where it redissolves dried food coloring to convert the sample into a brightly colored solution, which becomes visible when the sample fills the hydrophilic circular region in layer 7 (Figure 1c). The quantity of hydrogen peroxide is measured in this device by tracking the time required for the sample to pass from the top of the device to the bottom, which is established by the appearance of green color in layer 7.[14] The selectivity for the assay is provided by the selective oxidative cleavage of the aryl boronate in **1** via hydrogen peroxide,[6,15,16] although presumably other activity-based detection events could be employed if the aryl boronate is replaced with a substrate for another target analyte.[16,17]

Effect of the Quantity of 1 on the Limit of Detection (LOD). To render this type of assay general, we needed to increase the sensitivity of the system so that it could be applied to a variety of analytes that are present in samples at concentrations below low millimolar levels. In initial studies, we observed that there is a direct relationship between the concentration of hydrogen peroxide in the sample and the time required for the sample to flow through the paper-based device (Figure 2a). This behavior provides the basis for the

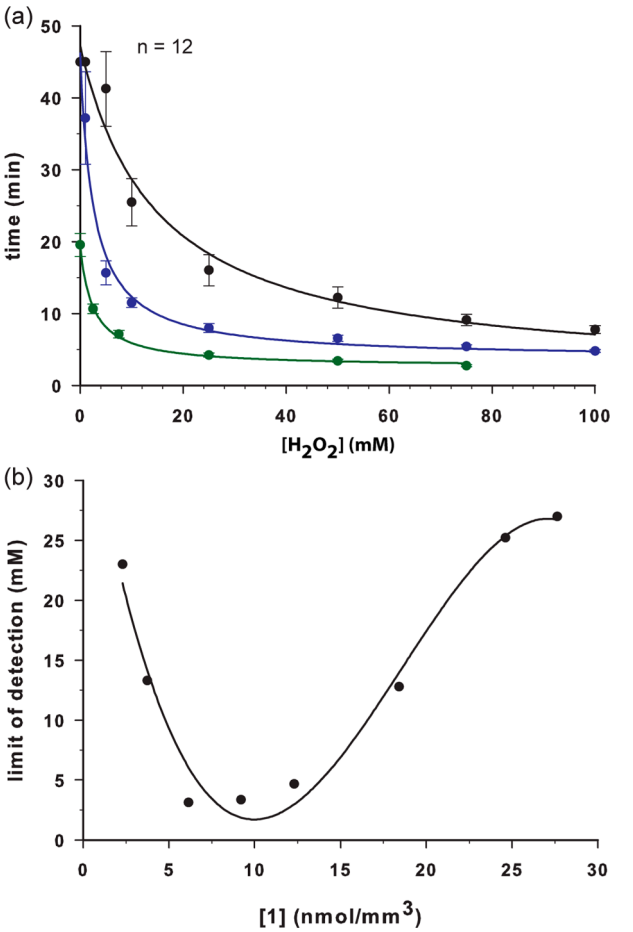

Figure 2. Relationship between **1** and the time required for a sample containing hydrogen peroxide to flow through the paper-based microfluidic device depicted in Figure 1. (a) Effect of the quantity of **1** on the flow-through time for the assay. The green data was acquired using 6.1 nmol of **1** per mm^3 of paper, the blue data using 9.2 nmol of **1** per mm^3 of paper, and the black data using 18.4 nmol of **1** per mm^3 of paper. The data points are the averages of 12 measurements, and the error bars reflect the standard deviations from these averages. The assay was stopped at 45 min, regardless of whether the sample had wicked through the device. (b) Relationship between the quantity of **1** per mm^3 of paper and the limit of detection for the assay. The limits of detection were calculated using graphs such as those in part a, where the linear region of the exponential response was used along with the equation LOD = $(3 \times \sigma_o)$/slope.

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quantitative assay, but it also offers an opportunity to increase the sensitivity of the method. For example, as revealed in Figure 2a, different quantities of **1** predeposited into layers 3 and 5 substantially affect the sensitivity of the assay: i.e., the green data were obtained from devices that contained 6.1 nmol of **1** per mm^3 of paper in layers 3 and 5, while the black data originates from devices that contained 18.4 nmol of **1** per mm^3 of paper. The limit of detection[18] for the former is 4.1× better than the latter (i.e., 3.1 mM H_2O_2 vs 12.8 mM), indicating that the quantity of **1** is critical for achieving the optimal limit of detection for the assay. In fact, this relationship between the quantity of **1** and the limit of detection for the model assay for hydrogen peroxide is parabolic, as indicated in Figure 2b, thus suggesting that a well-defined quantity of **1** must be present in the paper to convert into hydrophilic products upon reaction with hydrogen peroxide to allow the sample to pass through layers 3 and 5 in an appropriate time frame. With too much of **1** in the device, samples containing low concentrations of hydrogen peroxide flow through with rates equal to samples lacking hydrogen peroxide since the relative change from hydrophobic **1** into hydrophilic products is small. With too little of **1**, samples containing different low concentrations of hydrogen peroxide flow through equally quickly. Clearly the absolute quantity of **1** per volume of paper is important for maximizing the sensitivity of the assay, but so too is the relative quantity of **1** that converts to hydrophilic products upon reaction with the analyte. On the basis of this observation, we predicted that the sensitivity of the assay could be improved if we altered the magnitude of phase-switching provided by derivatives of **1** in response to the target analyte.

Chemical Strategies for Improving the Limit of Detection. We hypothesized that two different methods of altering the phase-switching molecule would increase the sensitivity of the assay. In the first approach (Figure 3a), we designed a derivative of **1** (i.e., compound **2**) that should degrade into hydrophilic products faster than **1**. In previous studies on controlled release reagents,[19] we found that addition of a methyl ether (labeled blue in Figure 3a) ortho to the benzylic leaving group on the benzene ring substantially accelerates the rate of quinone methide elimination reactions, which is the proposed degradation pathway for **1** and **2** to convert to hydrophilic products. We predicted that this quinone methide elimination reaction was the rate-limiting step in converting **1** into hydrophilic products during the assay. Therefore, by accelerating this quinone methide elimination reaction, we reasoned that more of hydrophobic compound **2** than **1** would convert to hydrophilic products in the time frame that it takes for (i) hydrogen peroxide to react with **2** and (ii) for the aqueous solution to pass through layers 3 and 5 in the device. If more of **2** converts to hydrophilic products than **1** within the time frame of flow-through, then less hydrogen peroxide would be needed to enable flow-through using **2** than **1**, and thus the sensitivity for the assay should increase.

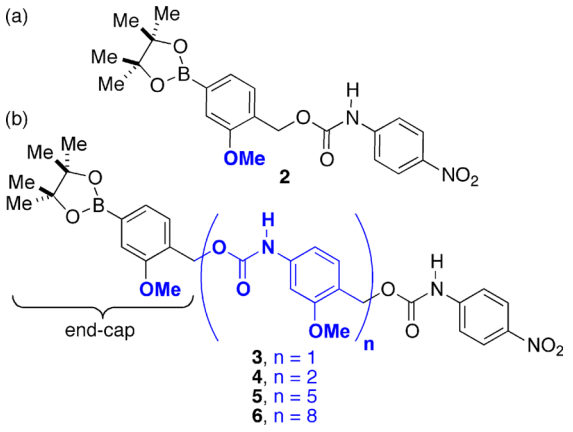

Figure 3. Hypotheses for increasing the sensitivity of the quantitative assay by replacing **1** with (a) a derivative that degrades faster than **1** when exposed to hydrogen peroxide, or (b) oligomers that provide a greater hydrophobic-to-hydrophilic change than **1** when exposed to hydrogen peroxide. In part b, the oligomers are designed to depolymerize once the end-cap (aryl boronate) is cleaved from the polymer and converted into a terminal phenol. The proposed mechanism of depolymerization is similar to the arrow pushing mechanism depicted in Figure 1d. The structural changes for **2**–**6** in relation to **1** are highlighted in blue.

In the second approach (Figure 3b), we predicted that oligomers modeled after **2** would provide a greater change in hydrophobicity than **2** by converting from a large hydrophobic molecule (an oligomer) to small hydrophilic products upon reaction with hydrogen peroxide, thus further increasing the sensitivity for the assay since the change in wetting properties will be amplified relative to **2**.[20] The proposed mechanism of response for the oligomers is as follows: oxidative cleavage of the aryl boronate in **3**–**6** would generate a phenol, which would initiate a cascade head-to-tail depolymerization reaction[21−23] through quinone- and azaquinone−methide-mediated pathways,[7] similar to the mechanism depicted in Figure 1d for compound **1**. We modeled the oligomers after **2** because we anticipated that the rate of depolymerization would substantially impact the limit of detection for the assays. Therefore, we included methyl ethers on each repeating unit to accelerate the rate of formation of azaquinone methide during the depolymerization reaction,[7] much like we included a methyl ether on **2** to increase the rate of formation of quinone methide.

Synthesis of Poly(carbamate) Oligomers. Compound **2** was prepared from 4-(hydroxymethyl)-3-methoxyphenylboronic acid pinacol ester and *p*-nitrophenyl isocyanate. Compounds **3** and **4** were prepared in a stepwise fashion, as described previously,[7] while oligomers **5** and **6** were prepared according to the route outlined in Scheme 1. This route involved a tin-catalyzed polymerization[8] of monomer **8**, where the length of the oligomer was controlled by polymerization time. The polymerization reaction was quenched by addition of the aryl boronate end-cap, and a post polymerization modification was used to append the *p*-nitrophenyl carbamate.

Effect of Reaction Rate on the Limit of Detection. After optimizing the quantities of **1** and **2** needed for this quantitative flow-through assay (Tables S9−S13, Supporting Information), we found that **2** provided a 17-fold improvement in the sensitivity for the assay compared to **1** (i.e., LOD = 1707 μM for **1** vs 103 μM for **2**). Thus, this result suggests that formation of quinone methide indeed is the rate-limiting step under the conditions of the assay.

Effect of Depolymerization on the Limit of Detection. Oligomers **3**–**6** also impart significant improvements in sensitivity to the assay compared to **1** and **2** (Figure 4). For example, after optimizing the quantity of each reagent for the flow-through assay (Tables S1−S38, Supporting Information), we found that addition of one repeating unit (i.e., oligomer **3**) improved the limit of detection 6× compared to **2** and 106×

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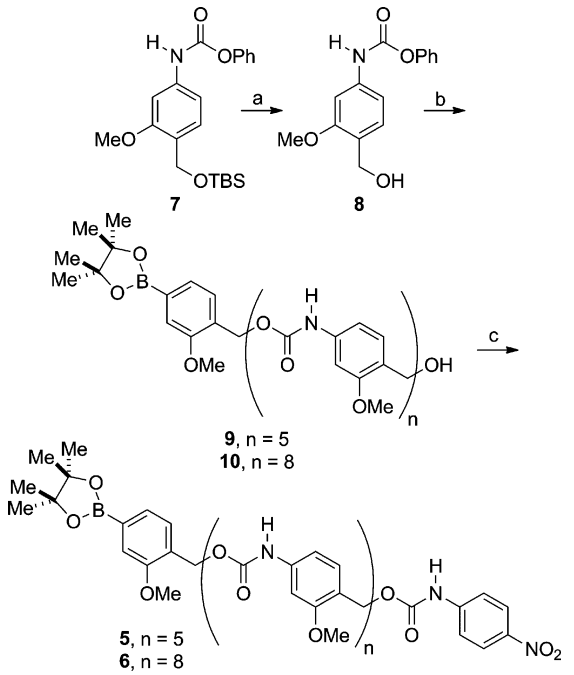
Scheme 1. Synthesis of Oligomers 5 and 6.[a]



[a]Reagents and conditions: (a)TsOH, THF−H_2O (87%); (b) (i) DBTL, (ii) 4-(hydroxymethyl)-3-methoxyphenylboronic acid pinacol ester, DMSO 110 °C (33% for **9**, 64% for **10**); (c) 4-nitrophenyl isocyanate, TEA, DMF (71% for **5**, 92% for **6**).

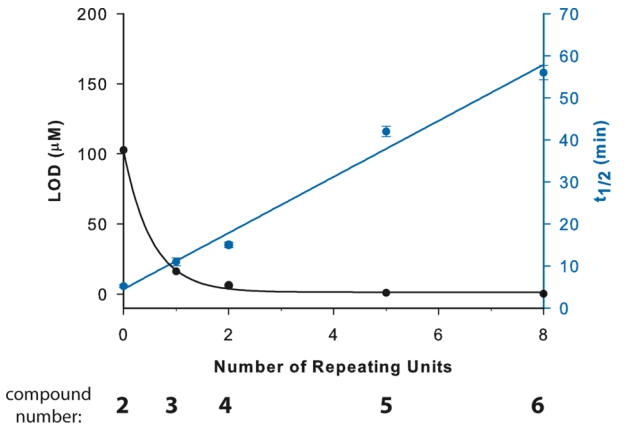


Figure 4. Limit of detection (LOD) (black data) and depolymerization half-lives (blue data) versus the number of repeating units in oligomers **2**−**6**. The half-lives were measured in a 5:4:1 solution of dioxanes−DMSO−H_2O using 20 equiv H_2O_2. Although the half-lives of carbamate oligomers depend on the polarity of the environment,[7] a similar linear relationship between depolymerization half-life and the number of repeating units in an oligomer likely exists in the environment associated with wet paper as well. The half-life data points are the averages of three measurements and the error bars reflect the standard deviations from these averages. The typical assay time for the limit of detection measurements was 25 min.

compared to **1**. In fact, a nearly linear relationship exists between limit of detection and the number of repeating units in the oligomer[8,24] until, presumably, the rate of hydrogen peroxide-induced depolymerization becomes competitive with the residence time of the sample within layers 3 and 5 of the device (Figure 4). Incomplete depolymerization would affect the magnitude of hydrophobic-to-hydrophilic phase-switching since the depolymerization intermediates (i.e., truncated oligomers) are expected to retain substantial hydrophobic character relative to the products of complete depolymerization. There appears to be a match in depolymerization time and flow-through time when approximately 2−5 repeating units are added to **2**, at which point less dramatic improvement in sensitivity is obtained when using longer oligomers that depolymerize more slowly than **4** (e.g., oligomer **6**, n = 8, has a LOD = 0.15 μM, whereas **5**, n = 5, has a LOD = 0.96 μM).

While we were unable to verify the depolymerization kinetics of **2**−**6** in the context of the paper-based microfluidic device,[25] solution-phase kinetics reveal the expected linear increase in half-life for depolymerization as the number of repeating units in the oligomers increases (Figure 4, blue data). This relationship between sensitivity of the assay, length of the oligomer, and depolymerization kinetics suggests that further improvements in sensitivity can be realized if longer polymers are used that have substantially faster depolymerization kinetics than the poly(carbamate) oligomers in Figure 3b.[26]

Modifications to the Device for Improving the Limit of Detection. Having established that both the magnitude of the hydrophobic to hydrophilic switch and the rate of depolymerization of the phase-switching reagent improve the sensitivity of the assay, we next explored whether the number of layers containing the oligomeric reagent within the device would impact the sensitivity and dynamic range for measuring hydrogen peroxide. On one hand, we reasoned that several layers containing small quantities of the phase-switching reagent might provide improved sensitivity for the assay by establishing repetitive hydrophobic to hydrophilic switching interactions between the sample and the oligomeric reagent. On the other hand, a single layer containing a large quantity of the phase-switching reagent (relative to a device containing multiple layers of the compound) might provide the maximum impact on controlling the flow of the sample in relation to the concentration of hydrogen peroxide in the sample.

To test these scenarios, we prepared devices that were similar to the device in Figure 1a, but with either one, two, or three layers of paper that were modified with oligomer **3** (Figures S1−S3, Supporting Information). We then characterized the relationship between the limit of detection and the total quantity of oligomer **3** in the devices to determine the minimum quantity of **3** needed to provide the lowest limit of detection for a particular design (Tables S14−S18 and S39−S49, Supporting Information). These experiments revealed that a device containing one layer of **3** provided a limit of detection for hydrogen peroxide that is nearly 5× better than a comparable device containing three layers of **3** (i.e., the LOD for one layer of **3** = 9.4 μM, while three layers of **3** = 46 μM). In fact, the limit of detection worsens by ∼2× every additional layer of **3** incorporated into the device. Likewise, the dynamic range for the assay worsens as the number of layers containing **3** increases. For example, the device containing one layer of **3** (Figure S1, Supporting Information) has a dynamic range of 9.4 μM to 1000 μM, whereas the device containing three layers of **3** (Figure S3, Supporting Information) has a smaller dynamic range of 46−250 μM. Clearly the best device design for improving the sensitivity of the assay involves providing a single layer where the magnitude of phase-switching is substantial, rather than providing small stages of phase-switching over several layers in a device.

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Combining Features to Improve the Limit of Detection. We now have the opportunity to combine the two approaches for improving sensitivity (i.e., improved reagents and device design) to ascertain the overall sensitivity limits for the current assay. Toward this end, we created a single layer device (Figure S1, Supporting Information) and determined the optimum quantity of **6** (the oligomer that provided the best LOD) needed to provide the lowest limit of detection for quantifying hydrogen peroxide in a sample. This revised assay requires only 1.9 μg of **6**, yet now provides measurements of hydrogen peroxide down to 6 nM, which is a LOD that is 500 000× better than our original assay reported in ref 6.

■ CONCLUSION

In conclusion, this article describes the use of depolymerizable poly(carbamate) oligomers[8−10,27] for improving the sensitivity of an assay that enables quantitative measurements of hydrogen peroxide by simply tracking the time required for a sample to pass through a 3D paper-based microfluidic device. The assay is selective for hydrogen peroxide based on the aryl boronate functionality in compounds **1**−**6**, quantitative (it has a LOD of 6 nM), does not require substantial input from the user, and is simple (it uses only paper, tape, and a poly(carbamate) oligomer that depolymerizes from an insoluble oligomer into water-soluble products in response to hydrogen peroxide).

Oligomers that depolymerize from head-to-tail in response to specific analytes offer a unique role in this assay, both as a hydrophobic-to-hydrophilic switch, and as a means of achieving signal amplification. This method of signal amplification[20] is unique among POC assays, both in the mechanism of amplification and in the realization that we cannot adjust the assay time to increase the sensitivity of the assay, as can be done with most signal amplification strategies. Instead, we increase the sensitivity by using oligomers that depolymerize in response to the target analyte, thus providing a greater hydrophobic-to-hydrophilic switch than is possible for a small molecule (e.g., **1**). Further improvements in sensitivity are expected if longer polymers are used (i.e., compared to **6**, which has 8 repeating units), but with the caveat that the polymers must depolymerize faster than the residence time of the sample in the device. Efforts to achieve this goal are currently in progress, as are studies to expand the scope of the method to measure analytes other than hydrogen peroxide.[16] Solutions to these aspects of sensitivity and selectivity will require thoughtful designs of new polymers to balance (i) the solubility of the polymer vs the monomer and (ii) the rate of depolymerization of the polymer,[28] particularly ensuring complete depolymerization in seconds to minutes of solid-state polymers.[29−32]

While an assay platform that offers further improvements in sensitivity will be useful, it is worth noting that the current limit of detection for hydrogen peroxide of 6 nM is sufficiently sensitive to measure hydrogen peroxide in rain and other sources of water where the presence of hydrogen peroxide is indicative of pollution.[33−35] Such an application may be ideally suited to a simple, inexpensive, rapid, "reader-less" quantitative assay that enables users to cheaply and easily track the quality of various sources of water for environmental analyses.

■ EXPERIMENTAL SECTION

Materials. All reactions were performed in flame-dried glassware under a positive pressure of argon unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (25−40 mmHg) at 30 °C. All reagents were purchased commercially and were used as received unless otherwise noted. 4-Nitrophenyl isocyanate was recrystallized from petroleum ether prior to use. *N*,*N*-Dimethylformamide, dimethyl sulfoxide, tetrahydrofuran, and triethylamine were purified by the method of Pangborn et al.[36] Flash-column chromatography was performed as described by Still et al.,[37] employing silica gel (60 Å pore size, 32−63 μm, standard grade). Thin-layer chromatography was carried out on silica gel TLC plates (20 × 20 cm w/h, F-254, 250 μm). Deionized water was purified by filtration and irradiation with UV light. The papers used were Whatman Chromatography Paper grade I and Boise Aspen 30 Printer Paper (92 brilliant, 30% postconsumer content), and the tape was Ace Hardware Plastic carpet tape (part no. 50106).

Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded using either a 300, 360, or 400 MHz NMR spectrometer at 25 °C, as indicated in the Experimental Section. Proton chemical shifts are expressed in parts per million (ppm) and are referenced to residual protium in the NMR solvent ($CHCl_3$ δ 7.26 ppm, $CO(CH_3)_2$ δ 2.05 ppm, or $SO(CH_3)_2$ δ 2.50 ppm).[38] Data are represented as follows: chemical shift, multiplicity (*s* = singlet, bs = broad singlet, *d* = doublet, *t* = triplet, *m* = multiplet and/or multiple resonances), integration, and coupling constant (*J*) in Hertz. Carbon chemical shifts are expressed in parts per million and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$ δ 77.0 ppm or $CO(CH_3)_2$ δ 29.8 and 206.3 ppm). UV/vis spectroscopic data were obtained using a six-cell spectrometer. Low resolution and high resolution mass spectra were acquired using mobile phases containing 5 mM ammonium formate. GPC data were acquired on a 300 × 7.5 mm, 3−100 μm particle size styrene divinylbenzene copolymer column using 1 mL/min *N*,*N*-dimethylformamide as the mobile phase. Molecular weights were calculated from low-angle and right-angle light scattering data. The system was calibrated using polystyrene standards.

Preparation of Compound 2. Triethylamine (52 μL, 0.38 mmol, 2.0 equiv) was added dropwise to a solution of 4-(hydroxymethyl)-3-methoxyphenylboronic acid pinacol ester (50 mg, 0.19 mmol, 1.2 equiv)[7] and 4-nitrophenyl isocyanate (26 mg, 0.16 mmol, 1.0 equiv) in tetrahydrofuran (2.0 mL). The reaction mixture was stirred at 23 °C for 4 h. The solvent was removed by rotary evaporation and the residue was purified by silica gel flash column chromatography (10% ethyl acetate in hexanes, increasing to 20% ethyl acetate in hexanes) to afford compound **2** as a white, amorphous solid (46 mg, 0.11 mmol, 67%): IR (cm^{-1}) 3313, 2977, 2360, 1738, 1600, 1549, 1508; ^{1}H NMR δ (360 MHz, $CO(CH_3)_2$) 9.46 (bs, 1H), 8.22 (d, 2H, *J* = 9.3 Hz), 7.82 (d, 2H, *J* = 9.3 Hz), 7.41 (d, 1H, *J* = 7.3 Hz), 7.36 (d, 1H, *J* = 7.4 Hz), 7.31 (s, 1H), 5.26 (s, 2H), 3.89 (s, 3H), 1.34 (s, 12H); ^{13}C NMR δ (360 MHz, $CO(CH_3)_2$) 157.6, 154.0, 146.4, 143.4, 129.3, 128.4, 127.8, 125.7, 118.6, 116.4, 84.6, 62.9, 55.8, 25.2 (there appear to be overlapping peaks in the aromatic region of the ^{13}C spectrum); MS (TOF MS AP−) 427.2 ($M - H^+$); HRMS (TOF MS AP−) calcd for $C_{21}H_{24}N_2O_7B$ ($M - H^+$) 427.1677, found 427.1657.

Preparation of Compound 8. *p*-Toluenesulfonic acid monohydrate (0.35 g, 1.9 mmol, 0.30 equiv) was added in one portion to a solution of compound **7** (2.4 g, 6.2 mmol, 1.0 equiv)[7] in 4:1 tetrahydrofuran−water (62 mL) under an atmosphere of air. The reaction mixture was stirred at 23 °C for 4 h. Ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate (10 mL) were added, each in one portion, and the layers were separated. The organic layer was washed with saturated aqueous sodium bicarbonate solution (1 × 50 mL) and was dried over sodium sulfate. The sodium sulfate was removed by filtration, the solvent was removed by rotary evaporation, and the residue was purified by silica gel flash column chromatography (20% ethyl acetate in hexanes, increasing to 60% ethyl acetate in hexanes) to afford compound **8** as a white, amorphous solid (1.5 g, 5.4 mmol, 87%): IR (cm^{-1}) 3540, 3470, 3269, 2963, 1727, 1615, 1547; ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.41−7.16 (m, 8H), 6.74 (d, 1H, *J* = Hz), 4.64 (s, 2H), 3.81 (s, 3H), 2.43 (bs, 1H); ^{13}C NMR δ (300 MHz, $CDCl_3$) 158.0, 151.8, 150.4, 138.4, 129.4, 129.1, 125.8, 124.3, 121.6, 110.2, 101.6, 61.5, 55.3; MS (Q MS APCI+) 256.1 ($M - OH^-$);

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HRMS (TOF MS AP+) calcd for $C_{15}H_{14}NO_3$ ($M - OH^-$) 256.0974, found 256.0967.

Preparation of Oligomer 5. Compound **8** (0.60 g, 2.2 mmol, 1.0 equiv) was added in one portion to stirring dimethyl sulfoxide (2.2 mL) at 110 °C. Dibutyltin dilaurate (0.26 mL, 0.44 mmol, 0.2 equiv) was added in one portion and the reaction mixture was stirred for 2.75 min at 110 °C. 4-(Hydroxymethyl)-3-methoxyphenylboronic acid pinacol ester (2.0 g, 7.6 mmol, 3.5 equiv) was added in one portion and the reaction mixture was stirred for 2 h at 110 °C. The reaction mixture was cooled to 23 °C and poured into 0 °C methanol (20 mL). A yellow precipitate formed that was washed using a solid phase washing vessel by adding methanol, bubbling N_2 through the solution at a vigorous rate (see the Supporting Information of ref 29 for a video of the bubbling rate) for 15 min, then draining the solvent. This process was repeated three times. The solids were dried under vacuum for 12 h to give oligomer **9** as an off-white powder (0.17 g, 0.15 mmol, 33%): ^{1}H NMR δ (360 MHz, $SO(CH_3)_2$) 9.90 (bs, 1H), 9.80 (bs, 3H), 9.65 (bs, 1H), 7.29−7.19 (m, 12H), 7.00−6.98 (m, 6H), 5.15 (s, 2H), 5.04 (s, 8H), 4.84 (bs, 1H), 4.39 (s, 2H), 3.84−3.70 (m, 18H), 1.29 (s, 12H). GPC: M_n = 1.2 kDa, M_w = 1.7 kDa, PDI = 1.44.

Triethylamine (0.12 mL, 0.86 mmol, 10 equiv) was added dropwise to a solution of oligomer **9** (0.10 g, 86 μmol, 1.0 equiv) and 4-nitrophenyl isocyanate (71 mg, 0.43 mmol, 5.0 equiv) in dimethylformamide (1.7 mL). The reaction mixture was stirred for 16 h at 23 °C, after which the solvent was removed by rotary evaporation. The residue was washed using a solid phase washing vessel with methanol (3×) followed by acetonitrile (2×). The solids were dried under vacuum for 12 h to give oligomer **5** as a peach-colored powder (0.81 g, 61 μmol, 71%): ^{1}H NMR δ (360 MHz, $SO(CH_3)_2$) 10.45 (bs, 1H), 9.90 (bs, 1H), 9.80 (bs, 4H), 8.19 (d, 2H, J = 9.3 Hz), 7.68 (d, 2H, J = 9.1 Hz), 7.40−7.21 (m, 12H), 6.99 (d, 6H, J = 8.3 Hz), 5.15 (s, 2H), 5.09−5.03 (m, 10H), 3.83−3.75 (m, 18H), 1.29 (s, 12H). GPC: M_n = 1.4 kDa, M_w = 1.7 kDa, PDI = 1.3.

Preparation of Oligomer 6. Compound **8** (0.60 g, 2.2 mmol, 1.0 equiv) was added in one portion to stirring dimethyl sulfoxide (2.2 mL) at 110 °C. Dibutyltin dilaurate (0.26 mL, 0.44 mmol, 0.2 equiv) was added in one portion and the reaction mixture was stirred for 5.0 min at 110 °C. 4-(Hydroxymethyl)-3-methoxyphenylboronic acid pinacol ester (2.0 g, 7.6 mmol, 3.5 equiv) was added in one portion and the reaction mixture was stirred for 2 h at 110 °C. The reaction mixture was cooled to 23 °C and poured into 0 °C methanol (20 mL). A yellow precipitate formed that was washed using a solid phase washing vessel with methanol (3 ×). The solids were dried under vacuum for 12 h to give oligomer **10** as a light yellow powder (0.30 g, 0.18 mmol, 64%): ^{1}H NMR δ (400 MHz, $SO(CH_3)_2$) 9.89 (bs, 1H) 9.78 (bs, 6H), 9.63 (bs, 1H), 7.41−7.19 (m, 18H), 7.03−6.95 (m, 9H), 5.16 (s, 2H), 5.04 (s, 14H), 4.82 (t, 1H, J = 5.6 Hz), 4.40 (d, 2H, J = 5.7 Hz), 3.94−3.67 (m, 27H), 1.30 (s, 12H). GPC: M_n = 1.3 kDa, M_w = 2.3 kDa, PDI = 1.73.

Triethylamine (0.16 mL, 1.2 mmol, 10 equiv) was added dropwise to a solution of oligomer **10** (0.20 g, 0.12 mmol, 1.0 equiv) and 4-nitrophenyl isocyanate (97 mg, 0.59 mmol, 5.0 equiv) in dimethylformamide (2.4 mL). The reaction mixture was stirred for 16 h at 23 °C, after which the solvent was removed by rotary evaporation. The residue was washed using a solid phase washing vessel with methanol (3×) followed by acetonitrile (2×). The solids were dried under vacuum for 12 h to give oligomer **6** as a peach-colored powder (0.19 g, 0.11 mmol, 92%): ^{1}H NMR δ (360 MHz, $SO(CH_3)_2$) 10.46 (bs, 1H), 9.91 (bs, 1H) 9.80 (bs, 6H), 8.20 (d, 2H, J = 9.1 Hz), 7.69 (d, 2H, J = 9.2 Hz), 7.38−7.22 (m, 18H), 7.01−6.99 (m, 9H), 5.16 (s, 2H), 5.09−5.04 (m, 16H), 3.85−3.76 (m, 27H), 1.30 (s, 12H). GPC: M_n = 1.7 kDa, M_w = 1.9 kDa, PDI = 1.2.

Procedure for Fabricating the Paper-Based Microfluidic Device. The paper was patterned using a wax printer according to the procedures described in refs 6 and12. The wax was melted into the paper by placing the patterned paper in an oven at 150 °C for 105 s. The devices were assembled according to the procedures in ref 11; the layout of the device is shown in Figure 5. The paper used for all layers of wax-patterned paper in the devices was Boise Aspen 30 Printer Paper (92 brilliant, 30% postconsumer content). Layer 2 contains a paper disk (2.5 mm-diameter ×180 μm thick) soaked in HEPES buffer (45 mM, pH 8.0) and dried. Disks were loaded with HEPES by adding 600 μL of HEPES buffer to 320 mg of disks and dried under vacuum. Disks were Whatman Chromatography Paper grade I paper fabricated using an Epilog Mini 24 Laser (CO_2 laser). Layers 3 and 5 were loaded with 0.25 μL of one of the hydrophobic detection reagents (i.e., **1**−**4**) dissolved in EtOAc (compounds **5** and **6** were dissolved in THF). A solution of a hydrophobic detection reagent was spotted using a Drummond 0.25 μL disposable micropipet. Layer 6 contains a paper disk soaked in green dye. The disks were loaded with the green dye by adding 600 μL of green food coloring (1:5 food coloring−deionized-water) to 300 mg of disks followed by drying the disks under vacuum. After assembly, the devices were pressed using a rolling pin, applying medium pressure.

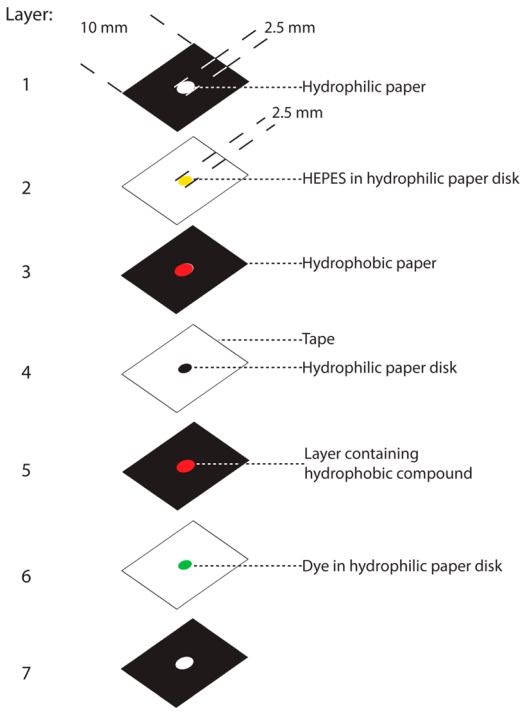

Figure 5. Expanded view of the device shown in Figure 1a. The device is 10 mm wide × 10 mm long × 0.9 mm thick.

Procedure for Measuring Flow-Through. We measured the time required for a sample to flow through the device in Figure 5 as follows: to layer 1 was added 8 μL of an aqueous solution of H_2O_2. A timer was started immediately upon addition of the sample to the device. The device was turned over so that layer 7 was visible. The flow-through time was recorded when the hydrophilic region of layer 7 had completely changed color. Fourteen replicate tests were performed for each concentration of H_2O_2 and the two highest and two lowest flow-through times were removed from the data set to account for errors arising from failures during the device fabrication procedure.

Procedure for Measuring Depolymerization Kinetics. *p*-Dioxanes (250 μL), dimethyl sulfoxide (190 μL), and phosphate buffered water (40 μL, 0.01 M, pH 7.1) were added to a 2 mL vial and mixed by swirling the solution. A solution containing the oligomer (10 μL from a 0.01 M solution in DMSO) was added to the vial and vortexed for 5 s. Hydrogen peroxide (10 μL from a 0.2 M solution in phosphate buffered water, 0.01 M, pH 7.1) was added and the combined solution was aspirated using a pipet. The solution was transferred to a quartz cuvette (500 μL, 0.1 cm path length) and the absorbance value at 385 nm was monitored continuously. Half-lives were calculated based on the relative quantity of released *p*-nitroaniline, using the method described in reference7.

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■ ASSOCIATED CONTENT

Supporting Information

Tables of primary data, effect of number of layers on sensitivity, calculation of limits-of-detection, depolymerization kinetics, and figures of NMR spectra and GPC traces. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(25) Because only ∼0.5 μg quantities of **2**−**6** are used in the paper-based microfluidic device, Raman spectroscopy was unable to distinguish background signal from paper from signal arising from the oligomers. Likewise, reflectance measurements targeting the color produced by the released *p*-nitroaniline upon depolymerization were unsuccessful due to the limited quantity of color produced during flow-through.

(26) A logical extension of this work would involve adding two methyl ether groups ortho to the benzylic leaving group on each repeating unit in the oligomer. In previous work focused on the controlled release of phenols, we demonstrated that incorporation of two methyl ethers enhanced the rate of release of phenol 35−fold compared to an analogous controlled release reagent that incorporated only one methyl ether.[19] In the context of carbamate oligomers, however, the benzylic leaving group is a carbamic acid rather than a phenol, and thus is a better leaving group. The consequence is that incorporation of two methyl ethers in carbamate oligomers caused substantial background decomposition that not only made their synthesis difficult, but also prevented their use in the assay.

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